

## Cryopreservation of Plant Germplasm: A vital tool for Genetic Resource Collections

### Plant germplasm types:

Seeds, Pollen, Explants, Spores

#### Germplasm stored in the freezer:

Orthodox seeds - most annual crops

#### Germplasm stored cryogenically:

##### Recalcitrant seeds -

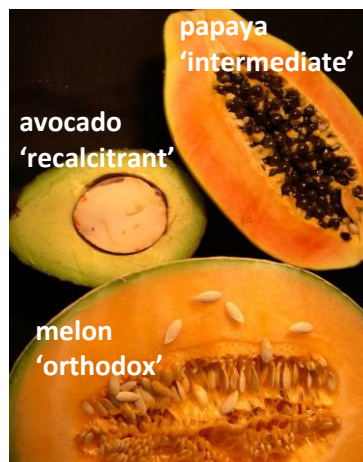
- tropical fruits (i.e., citrus, avocado, cacao, mango, lychee, rubber)
- iconic trees (i.e., oaks, buckeye, chestnut, & butternut)
- species of cultural significance (i.e., wild rice)
- species of conservation concern (i.e., yew)

##### Intermediate seeds -

- tropical and subtropical (i.e., coffee, papaya)
- many palm species
- grains with lipid having saturated fatty acids
- Some trees (i.e., aspen, cottonwood, elm)

##### Explants - growing shoot tips, dormant buds

- elite lines that outcross (i.e., fruit cultivars)
- seedless varieties (i.e., garlic)
- highly endangered populations



Fruit from the grocery store exhibiting seeds having different seed storage behaviors

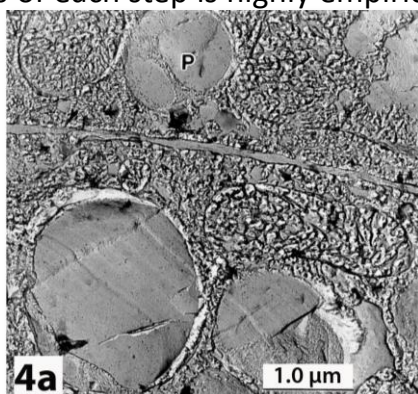


Shoot tips vitrified in cryoprotectants and photographed at -196°C

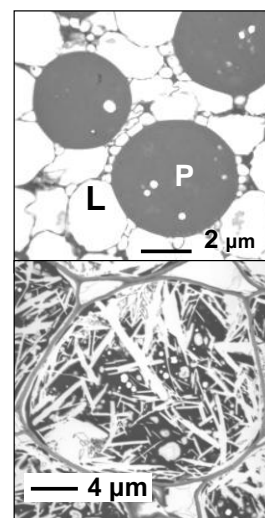
Cryopreserving plant germplasm requires surgical excision of totipotent cells, partitioning water within and outside cells, and forming a glass. Recovering germplasm must form normal plantlets. Currently, optimizing conditions of each step is highly empirical, which slows progress.



Micrografting citrus shoot tips promotes recovery



Intracellular ice isn't lethal if crystals are less than 0.5 μm



Optimum cooling to LN maintains cell integrity (top) that was lost when intermediate seeds were stored in the freezer (bottom)